ASEPTIC PROCESSING, VALIDATION OF

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INTRODUCTION

Aseptic processing is a widely used methodology in the health care industry for the preparation of sterile materials. The term aseptic processing as it is applied in the pharmaceutical industry refers to the assembly of sterilized components and product in a specialized clean environment. The clean environment may be a conventional human scale classified clean rooms or an environment engineered to further reduce the likelihood of contamination by reducing (or as much as is possible eliminating) direct human contact with the product and components being assembled "aseptically." The idea of sterile products manufactured aseptically is inherently contradictory, a demonstrably sterile product cannot be produced aseptically using even the most advanced technology available today. Nevertheless, on any given day millions of putatively sterile dosage form units are produced using aseptic techniques that in the literal sense are inadequate to achieve sterility. A sterile product is one that is free from all living organisms, whether in a vegetative or spore state. This is an absolute condition, something cannot be partially or nearly sterile, the presence of a single viable organism represents a failure of the product, and the systems (environment, equipment, and procedures) used to produce it. Asepsis, that state in which all aseptically filled sterile products are manufactured, cannot be established as "sterile." Asepsis is commonly defined as a condition in which living pathogenic organisms are absent.

Putting aside the classical definitions, one must consider the real difficulty in establishing an aseptic environment, let alone a sterile one. The practitioner is left with a insurmountable task, to somehow create an environment free of any organisms, but also one (with the exception of isolators) in which personnel must be present to perform critical functions. The problem is further compounded if it recalled that personnel are considered the single greatest source of microbial contamination in aseptic processing. Recent experiments have shown that personnel clothed in new, sterile clean room garments slough viable contamination at a rate of roughly one viable particulate to 10,000 nonviable particles. During slow deliberate movements with the best possible clothing, operators will slough particulate and viable organisms. Therefore, the probability of human borne microbial contamination being released in the conventional clean room is one over the course of any reasonably long operational shift. With this fact in mind, how then is one to accomplish a truly sterile or even aseptic environment? Especially when we must consider that many organisms that are normally nonpathogenic, can under certain circumstances become opportunistically pathogenic. Among those circumstances are a debilitated condition of general health in the patient, or, as is increasingly common, immunological insufficiency due to age or pre-existing condition.

Other than the obvious considerations of proper facility design, sterilization validation, and sanitization procedures (all of which are discussed elsewhere in this encyclopedia), the focus of attention must be on the personnel and the activities which they must perform. These actions are broadly termed, aseptic technique, and like any other human activity they can be accomplished in a variety of ways. In order to better understand aseptic technique, some general guidance and examples of good and bad technique can be used to delineate what should and should not be permitted.

The fundamental concept behind every aseptic processing activity is that nonsterile objects must never touch sterile objects. This is often accomplished by the establishment of a "sterile field" in which the core activities are performed. All of the surfaces of the gowned human operator must be always considered nonsterile. Nonsterile objects including the operators hands must never be placed between the source of the air and a sterile object. The operators' hands and arms must always be kept at a level beneath that of open product containers. Sterile components should under no circumstances be touched directly with gloved hands, a sterilized tool should always

used for this purpose. Since gloved hands and arms will enter the sterile field they must never touch walls, floors, doors, etc. Strenuous lifting and moving of tanks, trolleys, etc. must not be done by operators assigned to work within or near the sterile field, because the more strenuous the activity the higher the level of particle generation, and at least some of the particulate released by the operator will surely by viable microorganisms.

Some of the techniques to avoid include: reaching over exposed sterile objects to make adjustments beyond them; correcting a stopper feed problem with a gloved hand; touching face, eye shield, or any other nonsterile object with gloves; taking an air sample directly over open containers; continuously standing inside flexible partitions that mark the boundary of the sterile field; breaking up clumps of components with gloved hands. Each of these actions exposes the sterile objects to undue risk of contamination from the personnel. Certainly there are more ways to contaminate the "sterile field" than we can imagine. For this reason, the procedures used in and around the "sterile field" must be carefully defined and followed closely by all personnel. These procedures should follow the general principles outlined above and are evaluated in a media fill simulation and performed in an identical fashion during aseptic processing. It is beneficial to define in writing how each procedure is to be performed and train the operators in these exact procedures.

Worst Case

No discussion of aseptic processing validation can be considered complete without some mention of ''worst case.'' As initially defined by the FDA, worst case included consideration of numbers of personnel, temperature, relative humidity, and other aspects (1). This aspect has been adopted with some degree of modification by industry which has included some, but certainly not all of the FDA guidance. Some of the more common worst case situations which industry employs include: number of personnel, maximum hold time for containers and other items prior to filling, number and type of interventions performed. It is important to note that the determination of worst case conditions in a aseptic processing has been largely intuitive and highly subjective. Quantitative risk analysis is rarely if ever undertaken to establish and categorize actual modes of failure. Thus, worst case conditions for tests are established largely by precedence rather than actual data. The most significant worst case condition that is employed is the use of a microbiological growth media itself. Because the majority of aseptic formulations have either a preservative system and some products are inherently inhibitory or nonsupportive of microbial growth, the media represents a substantially more favorable environment for the survival of microorganisms. The protocol prepared for the aseptic processing validation effort should delineate where worst case type considerations have been incorporated into the experimental plan. Throughout this document, recommendations will be made to worst case assumptions where choices in the definition of the validation effort must be made.

PREREQUISITES

The validation of aseptic processing should be preceded by the formal validation of the various systems, which contribute to the sterility assurance of the materials to be produced. In essence that mandates that the facility, HVAC system, sterilization procedures for the product contact surfaces, equipment, components and product, sanitization/disinfection procedures for the suite, and personnel gowning. Merely listing the activities, which must be completed, serves to indicate the magnitude of the effort required to prepare for the aseptic processing validation effort. It is sometimes tempting to begin the validation of aseptic processing while these tasks are still underway, especially when one considers that it is universally accepted that the primary source of microbial contamination in an aseptic process are those activities which are performed by gowned personnel (Table 1).

It should be evident that virtually all of the items that top the list are either performed by or corrected by the human operator. The impact of the remaining factors is widely acknowledged to be of secondary consideration.

Table 1 Most likely sources of microbial contamination in aseptic processing[a]

1.	Personnel borne contaminants
2.	Human error
3.	Non–routine operations during aseptic process
4.	Assembly of sterile equipment prior to use
5.	Mechanical failure
6.	Inadequate or improper sanitization
7.	Transfer of materials within APA
8.	Routine operations during aseptic process
9.	Airborne contaminants
10.	Surface contaminants
11.	Failure of sterilizing filter
12.	Failure of HEPA filter
13.	Inadequate or improper sterilization

[a]From most to least likely. (From Ref. 2.)

Yet proceeding with the validation of aseptic processing before completing the validation of the supportive processes and systems raises the risk of failure unnecessarily and makes failure resolution well nigh impossible. Obviously, given the significance of human borne contamination as a risk factor, training and qualification of operators is a significant prerequisite to aseptic process validation. However, in an effort to move validation along quickly many firms do not emphasize training or even take short cuts with personnel education. Fortunately the various validations, which are required, are well documented in the literature and the practitioner should have no difficulty finding information on their execution (3).

REGULATORY AND HISTORICAL PERSPECTIVES

Aseptic processing activities are evaluated through process simulations in which a microbiological growth medium is utilized in the process in lieu of the product. The media is incubated after completion of the process to evaluate the procedures utilized. When utilized to evaluate aseptic filling, it is more narrowly defined as "a means of validating the aseptic assembly process that involves the use of a microbiological growth nutrient medium to simulate sterile product filling operations" (4). This technique was first applied in the late 1940s by Rhode, and incorporated into a World Health Organization guideline in the mid 1970s (5). In the late 1980's, the PDA developed one of the first guides to the execution of media fills for the evaluation of aseptic processing (6). Several years afterwards the U.S. Food and Drug Administration defined its aseptic processing requirements for the first time (7). Additional guidance has been developed by other regulatory and pharmacopeial sources, which have defined the required activities (8–12). The PDA latest guidance documents provide perhaps the most comprehensive source of information on process simulation tests (13, 14). The desire to evaluate aseptic processing activities other than sterile drug filling has made for some adaptation of the classical media fill test, and for this reason the term process simulation test has come into vogue to embrace a wider range of aseptic processing activities. Process simulation tests can be defined as a means for "evaluating an aseptic process employing methods that closely approximate those used for sterile materials using an appropriate placebo material" (15). As mentioned previously, central to the evaluation of an aseptic process is the inclusion of the required interventions which must be performed. Any process simulation that does not properly evaluate employee aseptic technique and does not fully consider the process human interface is technically invalid.

THE MECHANICS OF MEDIA FILL (PROCESS SIMULATION) EXECUTION

The conduct of aseptic processing validation ordinarily requires the use of a microbiological growth medium in lieu of the product. The PDA/PhRMA joint task force of Validation of Sterile Bulk Processes has outlined some process simulation methodologies which do not require the use of media (see later discussion on this subject), but aside from bulk applications process simulation testing has become essentially synonymous with media fill (16).

Media Sterilization

The execution of a media fill begins with the sterilization of the liquid media. This can be accomplished using either bulk sterilization of the liquid media in a large (glass or stainless steel) container or by filtration with a sterilizing grade filter. The choice of sterilization method is based on considerations of the volume of media required, growth promotion requirements, and filtration rate for the media. Provided that the media is introduced into the process at or before the point in which the production process being simulated becomes sterile, the choice of sterilization method is open. Whether the media be sterilized by filtration or by steam in bulk, there is no benefit to be gained from requiring that the sterilization method used be identical to that for the production materials. It is often suggested that a media fill test can be used to verify formulation bioburden control and process filter validation; however, this is not the case because as already pointed out the growth promoting and physical characteristics of media are far different from those of nearly all pharmaceutical preparations. Sterilization validation is established independent of the process simulation, and is the appropriate activity in which to confirm the appropriateness of the methods employed.

Manufacturing Activities

Once the sterile medium is in place the simulation can begin in which the aseptic processing steps are executed through the conclusion of the process. In many cases the simulation entails the filling of the product into its final container and explains its more commonly used name—media filling. To conclude however that media filling is all

that is required in the validation of aseptic processing ignores the many possible human interventions which can take place during the manufacturing and prefilling activities. Many sterile dosage forms require the execution of a significant number of complex aseptic manipulations after the materials become sterile. Suspensions, creams, ointments, implants, and liposome formulations are among the more common examples of processes where aseptic processing involves activities other than filling of containers. In the more ordinary production of sterile solutions there are manufacturing activities such as sampling and integrity testing of filters which can potentially expose sterile materials to contamination. Thus, a process simulation must include all of these prefilling activities in order to mimic the actions routinely performed in production of sterile materials. Understanding these additional requirements makes it evident why process simulation has come into vogue as a more appropriate description for this activity as opposed to more restrictive media filling. Evaluation of aseptic manufacturing activities can be achieved independently of the filling process using a variety of methods (17). If performed as part of an integrated activity with filling, the evaluation of the filled containers serves as verification of both aseptic manufacturing and filling practices.

The bulk production of sterile drug products such as antibiotics, corticosteroids, insulin, and certain biotechnology products requires that a number of processes be carried out under aseptic conditions. These processes can be evaluated in a manner adapted from those employed for aseptic filling processes. A joint PDA/PhRMA task force has developed the definitive guidance document on this subject (18).

Aseptic Filling

Media is filled into sterile containers using methods identical to those required for production of the sterile product. This activity has been the subject of numerous papers and several surveys of industry practice (19–25). Attention must be paid to the specifics of the aseptic filling process itself. The range of sterile dosage forms, which can be produced, encompasses variations in container type, container size, formulation, lot size, filling speed, and other aspects which should be embraced in the design of the validation program. Each of these must be given careful consideration in the definition of the program requirements and a sound rationale for the selection of the test conditions included in the validation protocol. Presented later are brief discussions of some of the issues to be addressed and some recommendations for execution. While the focus of these points is on activities during the simulated filling of containers, many of these are relevant to both the manufacturing of sterile bulk materials and the compounding of bulk sterile formulations.

Product-Related Considerations

Type of container

Sterile products are aseptically filled into a variety of containers including glass and plastic bottles, metal and plastic tubes, ampules, and plastic bags. The variety of these containers is matched by the variety of methods required to prepare them for use in the aseptic filling process. Where a filling line is used to fill different types of containers, the differences in sterilization and handling suggest that each type should be assessed independently of the others. Attempting to identify a worst case situation when different sterilization methods, handling issues, and sealing mechanisms are employed is tenuous at best. One container substitution that is always valid is the use of a container that allows the most effective and less intrusive reading of the results. For example, clear containers of identical dimensions should always be substituted for opaque containers provided closure feeding and/or sealing are not affected.

Type of product

The process simulation should encompass the procedures used in the entire filling process. Thus, for lyophilized product, the aseptic loading of the freeze dryer should be a part of the simulation. A suspension product that utilizes a recycle loop around the filling machine would be validated using an identical set-up even though the media being filled does not require such a set-up. Similarly, the validation of a powder filling process should include a placebo material passed through the powder handling system and then filled into the container. When placebo materials are used development tests to ensure that the ratio of placebo to media does not result in a failure of the placebo/media mixture to support microbial growth are necessary. Any special filling or handling activities that are specific to the product being simulated should be a part of the media fill. It is acceptable to add additional steps, such as liquid filling, to a powder fill process to allow for direct incubation of the filled units. Such additional steps may increase the potential for contamination of the filled units in the process simulation relative to the production filling process, but their inclusion is often unavoidable and represents an additional worst case challenge to the process. In some instances, the use of filling of control units, i.e., vials filled with liquid media but not the placebo powder may be beneficial as a means of assessing these

add-on activities, which are not a routine part of the aseptic filling process (26).

In complex processes, the process simulation may be divided into steps. Provided that the steps overlap, they can cover the entire process and allow for isolation of contamination to a specific portion of the overall aseptic process. This practice is employed commonly for freeze drying, where an number of vials can be filled and sealed without transfer to the freeze dryer as a means of distinguishing between contamination derived from the aseptic filling and contamination derived from the lyophilizer loading and freeze drying process (27). Detailed advice on the more common product types can be found in PDA's most recent document of process simulation testing (28).

Filling speed

The extremes of filling speed on the line should be considered in the validation planning. The use of the slowest normal filling speed may increase the potential for contamination ingress via deposition from the surrounding environment. The use of the fastest normal speed may increase the potential for human intervention by increasing the number of routine and nonroutine line interventions. The likely impact of fill speed will depend upon personnel population, proximity of personnel to the sterile field, and number of interventions. A rationale should be developed for the process simulation strategy chose. For example, in the initial validation of a filling line, one fill might be performed at the slowest speed, and two at the highest speed. In routine evaluation of the line, the speeds would be alternated.

Container size

The size of the containers being filled is viewed in a manner similar to fill speed. The largest container (often filled at the slowest speed because of its large fill volume) often has the largest opening, so the potential for microbial entry from the environment should be the greatest for that size. At the other extreme, the smallest container (often filled at the highest speed by virtue of its lower fill volume), represents the greatest handling difficulty. Smaller containers are generally more fragile, and less stable, and thus would be more prone to breakage and jamming in the equipment. Any container that presents additional handling steps or is more prone to breakage or instability should be included in the validation program, as it may represent a greater challenge to the aseptic process than either the largest or smallest container processed on the line. As such it may represent an additional worst case to be addressed.

Closure system

One of the more common differences between products is the closure system. Closure systems are selected for compatibility with the formulation, and in some cases differences in formulation may be create differences in handling difficulty. A stopper that is more prone to clumping or jamming in the tracks of the stopper bowl will necessitate additional interventions not present with other stoppers of similar size and thus would be considered worst case situations. There are a number of specialized closure systems designed to facilitate the delivery of an aseptically filled product. As these systems have sealed interstitial spaces where product contact can occur during administration of the drug product, the simulation procedure for these product configurations should include this space and media should be allowed to contact these surfaces during the incubation.

Fill volume

The volume of media filled into the containers need not be the routine fill volume for the container. It should be of sufficient volume to contact the container-closure seal surfaces and sufficiently large to allow for easy inspection of the filled units postincubation. Despite the lower fill volume, the speed of filling should match that used for the routine filling of the product being simulated. Smaller containers should not be over-filled as sufficient air must be available in the container headspace to support the growth of aerobic organisms and problems have been encountered where the liquid media essentially fills the entire container.

Filling Process Related Considerations

Filling lines

Considering the number of permutations of container, closure system, and other product attributes that must be encompassed in a process simulation program, it should be evident that only in the simplest of situations would a single set of media fills be adequate to provide coverage of all aseptic processes performed. Where multiple lines are present in the facility, each should be considered independently. Process simulation results of one line are not predictive of results on another because the contamination rate is primarily dependent upon human performance. Even identical equipment in two clean rooms designed to the same standard will not give uniform results unless the aseptic technique of the operators is at the same level of performance.

Duration of fill

The duration of the media fill is one of the more contentious issues. In general, media fills should be sufficiently long to include all of the required interventions. Using that requirement alone, a typical media fill might be at least 3–4 h long. Ideally a media fill should utilize more units than are in the product being simulated. This approach is normally followed for all batches up to 5000 units. As the number of units in batch increases current practice is to fill at least 5000 units, and increase the number as the batch size increases. For very large batches or long the campaigns common in blow/fill/seal or isolator systems, media fills interspersed with blank units (either empty or water filled) are used to maintain operating conditions during the simulation. Where this is done, media is filled before and after all planned routine and nonroutine interventions, and conversion to media is performed after any unplanned nonroutine interventions (see following section). Media filled units interspersed with blank units has been a technique used to validate processes that may run for several days. In these cases media is generally filled at the beginning to evaluate set up and then again at the end to evaluate the ability of the process to maintain asepsis for the full length of the longest approved campaign. Filling a large number of units as may be possible on a high speed filling machine is not a substitute for a realistic simulation of the process. A high speed filler could fill 5000 or more units in less than 15 min of filling time, yet that would hardly be considered an acceptable practice.

Interventions

In virtually all aseptic processing activities, operator interventions are required to complete the process. Understanding the types of interventions required and how they are incorporated into the validation program is essential to protocol development.

Aseptic assembly: The first interventions performed are those that prepare the equipment for the aseptic process. This entails the removal of sterilized materials and equipment items from the autoclave and transfer to the location where the aseptic processing activities will be performed. This is ordinarily followed by the assembly/preparation of the equipment for the process. Aseptic assembly in which sterilized parts are removed from protective materials, installed and adjusted in preparation for the aseptic process are perhaps the most potentially invasive of all of the activities which must be performed. The operator must be meticulous in their execution of these tasks to prevent the inadvertent contamination of product contact surfaces. Strict adherence to the principles of aseptic technique described earlier is essential. These interventions are a necessary part of every aseptic activity, and it is common to identify the first containers filled as they may be more indicative of potential problems with the aseptic assembly. For this reason, the validation program should include process simulations that include containers filled immediately after the set-up of the equipment.

Routine interventions: The execution of the aseptic process ordinarily requires a number of repetitive activities such as: product and component replenishment, weight checking, operator breaks, and environmental monitoring. Each of these is a required part of the process, and cannot be eliminated. They should be included in the process simulation and performed by the operators in a consistent fashion using defined methods and practices.

Nonroutine interventions: During the course of the aseptic filling process there may be instances where a nonroutine or corrective intervention is required. These usually occur in relation to difficulties with components or equipment aspects. Containers can break, jam in the conveyor, or fall over on a turntable. Stoppers can jam in the stopper track, clump in the bowl, or fail to seat properly. Problems with the equipment can include: weight adjustments, minor leakage, sensor failure, or rail adjustment. Each of these will require a corrective operation by the line operator to return the line to proper operation. Unlike routine interventions, these are not a required part of every process, but in order to assess their potential impact on the aseptic process the more prevalent of these should be included in every media fill. To the extent that these nonroutine interventions can be considered repetitive, i.e., weight adjustments, stopper jams, etc. their proper execution should be described in a procedure and adhered to by the operators. Nonroutine interventions may occur randomly or not at all during an aseptic filling process. To ensure that they are a part of the process simulation, they should be performed as if they were a required part of the simulation. Thus, even if the fill weights during the simulation are correct, the line should be stopped and an adjustment made to demonstrate the acceptability of the methods employed. Given the breadth of possible nonroutine interventions, which may be possible during a batch, it may not be possible to simulate all of them. The simulation protocol should address those the firm has identified as more commonly required. Firms should make a concerted effort to minimize the number and extent of these nonroutine interventions. It may be possible to reduce the need to perform them by improving component quality by tighter AQLs on incoming components, tighter controls on preparatory activities, repairs or upgrades to equipment, and similar activities. Such measures can contribute

substantially to the reliability of the process and patient safety.

New interventions: If during the conduct of a batch or a process simulation, the need for an intervention not previously evaluated in a process simulation may occur. The firm should allow for this eventuality and assess the intervention during its execution via supervisory observation. Further evaluation of the new intervention in a follow-up media fill should also be considered.

Documenting interventions: It can be beneficial to define in some detail the permitted interventions for a given aseptic process in an SOP. This practice eliminates any subjectivity regarding what is permitted, and also allows for the establishment of a defined method for performing the intervention. The SOP can be employed in training of the operators and used as guidance in both routine aseptic processing and process simulation. Documentation of routine processing and process simulation should include details on the interventions (routine and nonroutine) performed. The list of interventions required during routine filling can help to define the media fill program by establishing which are more common and should be given precedence in the process simulation. The required interventions can also be used as the initial justification for improvements to the procedures, components, and equipment used in the aseptic process. In the absence of such documentation during the process simulation, it is difficult to defend the acceptability of the intervention during routine processing. The use of video tapes as a means of both documenting interventions during media fills, and as a training tool for operators in the proper execution of an intervention is becoming more prevalent.

Environmental Considerations

Environmental monitoring

An aseptic processing activity is ordinarily supported by monitoring of the environmental air and surfaces in proximity to the process. The purpose of this monitoring is to confirm the acceptability of the environment during the process execution. There are a number of environmental sampling methodologies that are appropriate for this purpose each having particular advantages and disadvantages (29, 30). There are also a number of regulatory and pharmacopoeial references that delineate microbial levels considered acceptable for aseptic processing environments (31–34). Each of these documents has defined the microbial conditions under which aseptic processing operations should be conducted in a slightly different manner. This situation could prove problematic for those endeavoring to comply with all of the requirements simultaneously except that in the years since the FDA's guideline on aseptic processing was published, aseptic processing capabilities have improved substantially. The microbial limits which may have once proved so daunting are now routinely observed in the majority of aseptic processing applications. Twenty years ago in this industry it was in vogue to speak of the importance of identifying trends in environmental monitoring results. It is recognized today, that trends no longer exist and that the presence of a detectable microorganism in a critical location is a rare event (35). With such stellar performance near routine, some change in the paradigms relative to the performance of environmental monitoring relative to aseptic processing are necessary:

- The sensitivity of environmental sampling systems may be insufficient to detect microorganisms in critical environments with any degree of accuracy.
- Increased sampling in a effort to detect the already low levels of microorganisms is unlikely to be successful and can actually put the product at risk by increasing personnel incursions into the sterile field.
- Equipment and components have improved in quality to the extent, that environmental monitoring may be the most invasive intervention during an aseptic process (an undesirable situation).
- The detection of contamination of any type in an environmental sample from a critical environment, sterility testing or a filled container during a process simulation has become a rare event.

With these views in mind, environmental monitoring must be viewed in a new light. The following insights may prove useful to the practitioner:

- To paraphrase the Hippocratic Oath, the first rule of environmental monitoring should be "to do no harm." Sampling in a effort to detect microorganisms should not increase the potential for contamination of sterile materials.
- No amount of sampling could ever establish the acceptability of filled containers of sterile product.
- The sampling and enumeration of microorganisms is perhaps more prone to inadvertent contamination than the aseptic process itself.
- The identification of a recoverable organism in the environment is a random event and may have no relationship to the integrity of the "sterile field," or the sterility of the goods being produced. In fact, given the presence of human operators (including the individual performing the sampling) how could one not expect to find occasional contamination?

- There are no "smoking guns," establishing linkage between sterility test failure isolates, media fill contaminants and environmental isolates is extremely difficult.

Despite these somewhat negative perspectives, environments must still be monitored, and the levels of microorganisms controlled at levels that reconfirms the continued acceptability of the environmental conditions. Air sampling, using either active or passive sampling methods should be performed during the execution of the process. Surface sampling is best performed after the completion of the aseptic process to prevent the inadvertent contamination of product contact surfaces during the process. The vast majority of the samples taken should be devoid of contamination; however, the incidental detection of a recoverable organism from even product contact surfaces postprocess should not be cause for undue alarm. The sampling of the environment is also an aseptic process, and subject to its own flaws.

Personnel monitoring

The evaluation of microbial contamination on operating personnel is a necessary part of the overall program. Sampling should be considered from a perspective similar to that described above for environmental air and surfaces. In this context samples should be taken from hands and other gown surfaces only at the conclusion of the aseptic process. It is suggested that sampling of the operators be performed on every exit from the aseptic area. Unlike environmental air and surface samples, it is unrealistic to expect that all of these samples will be free of detectable organisms, nevertheless the individuals should be able to consistently meet the levels established for them. Individuals who demonstrate a repeated pattern of nonconformance with the expected microbial levels should be subjected to corrective measures. The actions taken could include retraining, aseptic processing reevaluation and gowning recertification (see following section). Personnel undergoing these corrective measures would be assigned duties outside the aseptic processing area until they have reestablished acceptable performance.

Personnel Considerations

Preparatory training

Some firms have adopted specialized aseptic processing exercises to evaluate and prepare personnel being introduced to aseptic processing activities for the first time. These tests can take the form of hand filling, media transfers, and other procedures designed to challenge the aseptic technique of the individual in the absence of the mechanical equipment. Only after successful completion of the hands-on manipulations would an individual be considered for further training as an aseptic operator. Whether this type of evaluation is performed or not, it is generally accepted that new personnel should actively participate in a media fill before they would be allowed to perform those same activities during a production batch. Additional lecture and demonstration type training of personnel is also necessary in aspects of microbiology, aseptic technique, gowning, equipment operation, and of course CGMP.

Gowning certification

Personnel assigned work in aseptic processing areas are ordinarily subjected to initial and periodic certification of their ability to gown in the prescribed manner. Gowning certification includes sampling of a variety of gown surfaces in addition to those normally evaluated during routine monitoring (36). For new personnel, this might be successfully performed three times before they are permitted access to the aseptic corp. Annual or semiannual sampling reconfirms that personnel are still able to gown properly. Gowning certification is generally extended to include other individuals, i.e., supervisors, maintenance workers, housekeeping personnel who must access the aseptic area, but who do not perform any activities related directly to the sterility of the products being manufactured.

Personnel participation

Virtually all aseptic processing operations require the active participation of human operators who are required to perform important tasks during the process in a manner that avoids the contamination of sterile materials, components, and surfaces. Their success in performing these tasks is assessed in the process simulation as they perform the routine and nonroutine interventions, which comprise their participation in the aseptic process. In order to establish that each of the operators is capable of successfully performing their duties their periodic participation in a process simulation is required. Set-up and line operators should be a part of not less than one process simulation per year. Individuals such as line mechanics and environmental samplers should be managed in a similar manner. Partici-pation requires more than mere presence in proximity to the aseptic process, it must include active execution of the interventions to which they are normally assigned. Supervisory staff and others, such as maintenance personnel who do not perform process related aseptic interventions should not be considered in the validation program.

One of the personnel concerns addressed by the FDA in its guideline on aseptic processing concerned the

maximum occupancy in the aseptic processing room (37). A simple means of accomplishing this is to designate a maximum occupancy for each room. Process simulations are then conducted at that level of staffing, and procedures are followed during routine operation in which a person must exit the room before another can enter whenever the maximum number of personnel are present in the room.

Where a firm operates on multiple shifts, the second and third shift should be included in the program as well to demonstrate the acceptability of their performance as well. Managing a large operation with large numbers of personnel, multiple filling lines and insuring that only personnel who have participated in a related media fill are allowed to perform aseptic processing can be a complex task.

Media Considerations

Media selection

The choice of a growth medium for use in the process simulation requires consideration of the organisms expected to be found in the environment, with emphasis on human derived contaminants from the operating personnel. With this in mind, the conventional choice is a general purpose medium capable of growing a wide range of common aerobic microorganisms (38). The usual choice is Soybean–Casein Digest Medium, also known as Tryptic Soy Broth, the same medium used in sterility testing. In some instances, other media might be appropriate. If filling is performed in an isolator under a total nitrogen environment, then an anaerobic medium such as Fluid Thioglycollate Medium might be more appropriate for the purposes of the simulation.

Use of anaerobic media

The sterility testing of parenteral products includes testing with both aerobic and anaerobic media. When media fills became common in industry during the mid-1970s the use of anaerobic media was considered a standard part of every aseptic processing validation program (39). Operational experience over recent years has indicated that the difficulty in establishing a truly anaerobic environment on a ordinary manned filling line are such that their execution is no longer common (40). Recognition that the predominant source of microorganisms in a clean room are personnel, the conduct of anaerobic media fills for other than special circumstances is unwarranted. Furthermore, anaerobic media fills are not required by any current regulatory body, as they also recognize that the focus of the effort should be on human borne contamination which can survive in air.

Media incubation conditions

An area of particular divergence in industry practice in the execution of media fills is that of incubation conditions (41). Cogent arguments can be made for incubation at a variety of temperatures. Some firms use only a single temperature in the range of 20–35°C. Other firms have chosen to incubate for 7 days at 20–25°C, and then move the filled containers to 30–35°C. An almost equal number have chosen to incubate for 7 days at 30–35°C, and then move the filled containers to 20–25°C. The lack of consensus suggests that the selection of incubation conditions is likely a minor concern. The suitability of the conditions employed, whatever they might be is established by the conduct of growth promotion studies (see following section). The one constant in this area is the duration of the incubation period which is almost universally set at 14 days (42).

Media growth promotion

The central issue in any discussion of appropriate media to use, the need to perform anaerobic media fills, selection of incubation conditions and the one that supports all of the decisions made in these areas, is the growth promotion studies. These establish that the medium used in the process simulation can successfully support the growth of microorganisms. Conventional practice is to test the media against a panel of organisms such as *Bacillus subtilis, Candida albicans, Pseudomonas aeruginosa, Staphylococcus epidermidis,* and *Aspergillus niger.* The use of one or perhaps two of the most common environmental isolates in the growth promotion studies is highly recommended as these may be the most likely to be encountered in a contaminated unit. Failures of the challenge organisms to grow voids the media fill and requires a repeat of the media fill. In media challenges where a powder is added to the media, studies demonstrating acceptable growth promotion are required (43). Process simulations using media performed in antibiotic product facilities will sometimes require the addition of inactivating enzymes to the media in order to obtain acceptable growth promotion results as even trace amounts of antibiotics can inhibit the growth of some organisms.

ACCEPTANCE CRITERIA

Over the last 20–30 years, nothing has proven more controversial in the validation of aseptic processing than the selection of a acceptance criterion to use. The first regulatory requirements for media fills was published by

WHO in the 1960s and had a criterion of 0.3%. Thus, a media fill of more than 1000 filled units would be considered acceptable if no more than three of those units were found contaminated after incubation (44). This limit lasted until the early 1980s when PDA prepared its first guidance on the validation of aseptic processing (45). The PDA document drew upon what was at that time the first survey on aseptic processing practice which had been conducted by PMA, and suggested a criterion of 0.1% of the units filled (46). This suggestion was at the time considered quite radical at the time as it seemingly raised the bar substantially. Interpretations of limit definition were made by Ronald Tetzlaff, at that time an employee of the Food and Drug Administration (47, 48). These drew upon suggestions made in PDA's second document on aseptic processing validation where a Poisson distribution was used to project the contamination over a large batch (49). FDA's first official statement on acceptance criteria for process simulations was provided in their 1987 aseptic processing guideline (50). The FDA guideline stated, "Test results should show, with a high degree of confidence, that the probability of a product becoming contaminated during aseptic processing is very low. In general, test results showing a probability of contamination of not more than one in 1000 are acceptable." With the publication of this document, PDA's 1980 limit had been echoed by a regulatory agency, and this limit remains FDA's current official position. PDA continued its work on aseptic processing through the conduct of industry surveys in 1986 and again in 1992 (51, 52). These efforts established that industry performance was continually improving and that 0.1% was almost universally accepted by industry on a global basis. The Parenteral Society (TPS) developed its first guidance on the validation of aseptic processing, and this placed greater emphasis on statistical treatment of the acceptance criteria than prior documents (53). This document profoundly influenced a ISO document that was then in preparation and a second controversy was born (54). The emphasis placed on statistics in both these documents made many uncomfortable. While mathematically correct the extension of the acceptance criterion tables to include larger numbers of filled units created an awareness of the statistical approach that had not existed previously. Inherent in the statistics, as the number of units filled in a media fill increased, the allowed number of contaminated units increased as well. While one contaminated unit in 1000 containers seemed acceptable, it is statistically equivalent to 10 contaminated units in 16,970 units. This seemed to many as an unacceptably large number of positives units in any size batch. Official EU guidance was provided for the first time in 1996 with the publication of their annex 1 on sterile medicinal products (55). The annex suggested that, "The contamination rate should be less than 0.1% with 95% confidence level." This was provided without elaboration, thus while the limit was clearly statistical the implications of the TPS and ISO efforts were not addressed. The clearest response to the TPS and ISO statistical data treatment was developed by the PDA (56). The PDA guidance states the following: "Despite the number of units filled during a process simulation test or the number of positives allowed, the ultimate goal for the number of positives in any process simulation test should be zero. A sterile product is, after all, one that contains no viable organisms." The PDA that originally introduced the Poisson approach for evaluation of media fills, has revised its perspective, and now believes that statistical treatment of the data is invalid. The Poisson method is appropriate for the evaluation of a low incidence of a random event, which is no longer believed to be consistent with how sterile materials become contaminated during aseptic processing. When first introduced it was assumed that contamination in an aseptic process could be derived from a variety of sources, and that any statistical approach that addressed that possibility as a random event was considered appropriate. With the execution of many more media fills, substantial improvements in facilities and equipment, and an increased awareness of the human contribution to microbial contamination views have changed substantially. It is now a widely held belief that contamination in aseptic processing is the result of human derived contamination resulting from improper technique in the execution of a required intervention. Thus, there is nothing random about the contamination, its source is known and its elimination certainly more possible than ever. This PDA view represents the latest thinking relative to acceptance criteria. The tenants of PDA's latest effort are provided in Table 2.

Additional publications which have provided acceptance criteria for aseptic processing validation have been prepared by both CEN and PIC (58, 59). These have attempted to reconcile the differences between the ISO document and the PDA guidance. The essence of these documents is the following. "Ideally the contamination rate should be zero. However, currently the accepted contamination rate should be less than 0.1% with a 95% confidence level." This appears to be an effort to have it both ways, and it is unclear whether the two perspectives can really be reconciled so easily.

The most recent industry survey on aseptic processing was published by PDA in 1997 (60). It attempted to address the statistical nature of the limits as actually practiced, however the response to questions in that area are inconclusive. It did include evidence that several firms

Table 2 PDA views on aseptic processing validation acceptance criterion

The test methodology must simulate the process as closely as possible.
Rationale for the chosen methodology and limits must be justifiable and documented.
Test methodology should be sensitive enough to confirm a low process simulation test contamination rate, and the selected limit must be routinely achievable.
Any positive unit indicates a potential problem, regardless of run size. All positives should be identified and should result in a thorough, documented investigation.
Process simulation test contamination rates approaching zero should be achievable using automated production lines in well-designed aseptic processing facilities, blow-fill- seal and form-fill-seal and in isolator-based systems.
Processes conducted in older facilities or employing considerable product handling or manual operation may not be capable of achieving near-zero contamination rates. Nevertheless, such processes must be capable of a process simulation test contamination rate not exceeding one in 1000 when 3000 units are filled.
For batch sizes smaller than 3000 units, process simulation tests should at least equal the batch size. No positives should be allowed due to the low sensitivity of small runs.
When more than 3000 units are filled, caution should be used when deciding to increase the allowable number of positives based on arithmetic extrapolation.

(From Ref. 57.)

had adopted acceptance criteria tighter than 0.1%, suggesting that another round of acceptance criterion definition might be in the offing. The most recent commentary on acceptance criteria is that provided by USP in draft chapter, 1116 (61). The USP expanded upon PDA's 1996 position, and established ever tighter requirements, "The goal is zero contamination. In an individual run not more than one positive unit in 5000 filled units. In a series of three media fills, two of the three media fills should have no contamination present."

Perhaps the simplest means of establishing an acceptance criteria (and perhaps to define one's entire program) is to follow the pack, and develop a firm's entire aseptic processing validation program based upon the latest survey information (62). What is certainly clear is that aseptic processing performance has improved substantially over the last 20 odd years and that firms should monitor their practices against their peers and regulatory expectation on a continuing basis. What was acceptable in the past is not acceptable today, and there can be little doubt that further tightening of the criteria can be expected in the future.

Technological Advances

The production of sterile products has benefited from at least two novel production methods: blow-fill-seal and isolation technology. Each of these can offer a substantial reduction in the amount of operator interaction with sterile materials. In blow-fill-seal (and the closely related form-fill-seal), the product container is created only seconds before it is filled and sealed. This has distinct advantages over more conventional filling where containers are exposed to the manned aseptic filling environment. A number of process simulation studies have established that the blow-fill-seal method is capable of protecting the contents of the sealed container to a greater extent than a ordinary clean room (63–65). Isolation technology enjoys similar results using an entirely different approach in which personnel are removed from the operating environment (66, 67). Isolation technology, which is a direct evolution of glove boxes, places operating personnel outside a sealed (physically or via an air pressure differential) enclosure in which the aseptic process is conducted. The enclosure can be treated with a sterilizing gas which can render the interior surfaces free of microorganisms. This treatment when combined with the elimination of direct personnel presence in the aseptic environment, makes the isolator perhaps the ideal tool for sterile drug production (68).

STERILITY ASSURANCE FOR ASEPTIC PROCESSING

To this point in this effort, the validation of aseptic processing has been described as closely related to media filling or process simulation. In reality the relationship between simulation and routine production is not a direct one. A completely successful media fill program does not establish the sterility of anything other than itself. The next lot, or for that matter the previous one, may be sterile or not. The absence of contaminated units in a media fill merely demonstrate that the facility, personnel, and procedures are capable of preventing contamination in that media fill. Demonstrating that capability for routine

filling is quite a different thing, and at the present time cannot be accomplished with other than a destructive sterility test of every filled unit. Ultimately the practitioner can only infer that because media fills are successful, that similar success is also possible during routine production. There is a common misunderstanding that the 0.1% or maximum of one in 1000 units is a sterility assurance level. That is most definitely not the case, it is nothing more than a maximum allowable contamination rate during the process simulation. There is no accepted means for establishing the sterility assurance level of aseptically filled products, it might be termed the "holy grail" of sterile production. We perform media fills to demonstrate a capability, and with significant limitations we infer from that effort that we can produce sterile drug products using aseptic processing. At the present time, this is perhaps as close as we can get to the "validation of aseptic processing." Despite this most basic of constraints, media fills represent the only means of even approximating what occurs when aseptic processing is performed. Demonstrating the capability of producing sterile products will have to be sufficient for industry needs.

CONCLUSION

Demonstrating success with aseptic processing requires process simulation studies closely matching the routine production activities. As the range of sterile products manufactured by aseptic processing is quite extensive, this mandates that the practitioner be prepared to adapt the general guidance provided in this effort to their particular situation. The more closely the simulation matches the production activities, the clearer indication that success with the simulation means success in routine operation. A well founded process simulation program affords the firm confidence in their routine operation that cannot be obtained by any other means.

It is important to consider however, that at the current level of technology, particularly manned aseptic processing, uncertainty is an inherent feature. The fact that process simulation tests are merely a snapshot in time has been recognized since media fills became a standard feature of aseptic process validation in the late 1970s. Guaranteeing safety to the end user is not as simple as successful media fill tests, "good" environmental monitoring results, and successfully completed sterility tests. Even when these data appear satisfactory, uncertainty exists. Although human nature detests uncertainty, particularly in fields where numerical values are widely stated, scientific rationality requires us to recognize that we cannot test or monitor uncertainty away. Only by thorough training, supervision and reduction of human borne contamination hazards can the likelihood of contamination be controlled. Fortunately, it appears certain from the absence of data to the contrary that aseptically produced health care products are very safe when produced in accordance with current industry standards. Certainly as the industry process capabilities continue to evolve our products will become safer still, and perhaps someday the uncertainty associated with aseptic processing will be so low that we can consider these products truly sterile.

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